<div align="center">

N THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

</div>

IN RE: WELSH, Michael J. et al.	)
	) APPEAL NO. _____
SERIAL NO: 10/659,467	)
	)
FOR: NOVEL COMPOSITIONS AND	)
METHODS FOR MODULATION OF	)
THE ACID-SENSING ION CHANNEL	)
(ASIC) FOR THE TREATMENT OF	)
ANXIETY AND DRUG ADDITION	)
	) BRIEF ON APPEAL
FILED: September 10, 2003	)
	)
GROUP ART UNIT: 1647	)
	)
ATTORNEY DOCKET NO: P05405US01	)
	)
CONF NO.: 6078	)

To the Commissioner of Patents and Trademarks
Mail Code Appeal Brief - Patents
P. O. Box 1450
Alexandria, VA 22313-1450

Dear Sirs:

      Please enter the following Brief on Appeal into the record.

<div align="center">

===
CERTIFICATE OF MAILING/TRANSMISSION

</div>

I hereby certify that this correspondence is, on the date shown below, being:

EXPRESS MAILING	ELECTRONIC
❑ deposited with the United States Postal Service with sufficient postage as Express mail in an envelope addressed to the Commissioner for Patents, Mail Stop Appeal Brief – Patents P.O. Box 1450, Alexandria, VA 22313-1450 Express Label No. _____	☑ transmitted by electronic to the Patent and Trademark Office, using the EFS

Date: 4-20-09

_____
JILL N. LINK

TABLE OF CONTENTS

I.   INTRODUCTION

This is an appeal of the Final Rejection dated August 20, 2008, finally rejecting claims 1-4, 24, 25, 30 and 31.

II.   REAL PARTY IN INTEREST

The real parties of interest in the present application are the University of Iowa Research Foundation of Iowa City, Iowa, and the United States Department of Veteran Affairs, Washington, D.C., the Assignees of record for this application. The assignments have been recorded at Reel 015087 and Frame 0006 on September 7, 2004 and at Reel 015086 and Frame 0989 on September 7, 2006 by the inventors Michael J. Welsh and John A. Wemmie.

III.   RELATED APPEALS AND INTERFERENCES

There are no related appeals or interferences at the time of this filing.

IV.   STATUS OF CLAIMS

Claims 1-4, 24, 25, 30 and 31 are pending. Claims 1-4, 24, 25, 30 and 31 have been rejected by the Examiner under 35 U.S.C. § 112, first paragraph, for both lack of enablement and written description. Claims 5-23 and 26-29 have been withdrawn from consideration as they are drawn to non-elected inventions. The Amendment After Final filed November 4, 2008 intended to cancel claim 4 of the application and inadvertently withdrew the claim. Therefore, claims 1-3, 24, 25, 30 and 31 are currently on appeal.

V.   STATUS OF AMENDMENTS

An Amendment After Final was submitted on November 4, 2008 amending claims 1, 24-25 and 30-31 and withdrawing claim 4. The Examiner's Advisory Action was mailed December 4, 2008 where the Examiner entered the amendments set forth in the Amendment After Final. A Notice of Appeal was timely filed on April 20, 2009.

VI.   SUMMARY OF CLAIMED SUBJECT MATTER

A.   Overview

The present invention relates to methods of treating central nervous system (CNS) disorders that are attributable to a variety of etiologies, including neurotransmitter system dysfunction. (Specification p. 1, lines 20-21). The claimed methods relate to the delivery of acid-sensing ion channels (ASICs) antagonists to the ASIC of a human in need of treatment with such ASIC antagonist. (Specification p. 11, lines 5-9). The disruption of ASIC on H+-evoked currents in the brain results in methods of treatment for anxiety disorder of post-traumatic stress disorder (PTSD) and improved fear responses. (Specification p. 2, lines 16-20).

B.   Independent claim 1

Independent claim 1 relates to a method for treating post-traumatic stress disorder. (Specification p. 5, line 30 through p. 6, line 7; p. 6, lines 13-17; p. 7, lines 21-25). The method comprises administering to a patient in need of treatment for post-traumatic stress disorder, a therapeutically effective amount of an acid sensing ion channel (ASIC) antagonist

2

and a pharmaceutically acceptable carrier. (Specification p. 7, line 30 through p. 8 line 4). The ASIC antagonist used for the treatment of post-traumatic stress disorder causes improved fear responses. (Specification p. 35, lines 1-14).

C.   Independent claim 24

Independent claim 24 relates to a method for treatment of a disease state associated with increased pH to improve fear responses. (Specification p. 2, lines 16-20; p. 11, lines 5-9). The method comprises administering to a patient a therapeutically effective amount of an acid sensing ion channel (ASIC) antagonist and a pharmaceutically acceptable carrier. (Specification p. 5, line 30 through p. 6, line 7; p. 6, lines 13-17; p. 7, lines 21-25; p. 7, line 30 through p. 8 line 4). The ASIC antagonist used for treating the disease state is capable of improving fear responses. (Specification p. 35, lines 1-14).

D.   Independent claim 30

Independent claim 30 relates to a method for treating a CNS disorder characterized by a change in extracellular pH in the amygdala. (Specification p. 2, lines 4-5; p. 12, lines 9-18). The method of treatment comprises inhibiting an acid-sensing ion channel 1 (ASIC1) to improve fear responses in a patient in need of such treatment. (Specification p. 5, line 30 through p. 6, line 7; p. 6, lines 13-17).

VII.   GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

A. Whether claims 1-3, 24, 25, 30 and 31 are unpatentable under 35 U.S.C. § 112, first paragraph, for lack of enablement.

B. Whether claims 1-3, 24, 25, 30 and 31 are unpatentable under 35 U.S.C. § 112,

first paragraph, for lack of written description.

VIII. ARGUMENT

A.   Claims 1-3, 24, 25, 30 and 31 are enabling under 35 U.S.C. § 112, first paragraph and are therefore patentable

1.   Legal Standard for Enablement

In rejecting claims under 35 U.S.C. §112, first paragraph, as lacking a specification

that fails to provide an enabling disclosure of the claimed invention, the test to apply is

whether one reasonably skilled in the art would be able to make or use the invention based on

"the disclosures in the patent coupled with information known in the art without undue

experimentation." *U.S. v. Telectronics, Inc.*, 857 F.2d 778, 785 (Fed. Cir. 1988). The Federal

Circuit has clarified that patent applications need not teach, but rather should preferably omit,

that which is known in the art and therefore unnecessary for purposes of enablement under 35

U.S.C. §112, first paragraph. *Id.* Further, Patent Office practice sets forth in the MPEP that if

the statement of utility contains a connotation of how to use, and the art recognizes that

standard modes of administration are contemplated, then 35 U.S.C. § 112 is satisfied. MPEP

§ 2164.01(c).

2.   The Claims in Issues are Legally Enabled for the Treatment of PTSD, Diseases with Increased pH and CNS Disorders Characterized by Changes in Extracellular pH with ASIC Antagonists in a Variety of Routes of Administration

The Examiner recognized in the Advisory Action dated December 4, 2008 that the claimed invention overcame the enablement rejections under 35 U.S.C. §112, first paragraph, in part. Specifically, the Examiner stated that claims 1-3, 24, 25, 30 and 31 are enabled for treatment of fear conditioning or PTSD with the ASIC antagonist PcTx administered directly to the brain. The Examiner further stated that the evidence reviewed indicates that administration of PcTx in mice demonstrates a reduction of fear conditioning by direct injections to the brain. However, the Examiner maintained the enablement rejections under 35 U.S.C. §112, first paragraph, with regard to the claimed invention of methods of treatment for PTSD, disease states associated with increasing pH in order to improve fear responses and CNS disorders characterized by changes in extracellular pH in the amygdala with the administration of an ASIC antagonist.

According to both legal precedent and Patent Office practice, the originally-filed specification directs and enables a person skilled in the art to which the claimed invention pertains, or with which it is most nearly connected, to make the invention commensurate in scope with the appealed claims. *Telectronics,* 857 F.2d at 785; MPEP § 2164.01(c). The appealed claims present an invention commensurate in scope with the enabling written description requirement as set forth by the Examiner during prosecution of the claimed invention. *See* MPEP § 2164. The specification provides the written description for the claimed invention which the Examiner improperly determined to be non-enabling; therefore, a review of this back-drop against which the claimed invention is measured should be properly considered from the viewpoint of a skilled artisan in the art to which the claimed

5

invention pertains. *CFMT, Inc. v. Yieldup Int'l Corp.*, 349 F.3d 1333 (Fed. Cir. 2003). In

CFMT the Federal Circuit reiterated that it is unnecessary to "enable one of ordinary skill in

the art to make and use a perfected, commercially viable embodiment absent a claim

limitation to that effect." *Id.* at 1338.

Here, the specification is enabling under 35 U.S.C. §112, first paragraph, for three

reasons. The specification sets forth for skilled artisans the: (1) various conditions requiring

treatment under the claimed methods (including PTSD, disease states associated with

increased pH in order to improve fear responses and CNS disorders characterized by a change

in extracellular pH in the amygdala), (2) ASIC antagonists to be administered under the

claimed methods, and (3) routes of administration for such methods.

First, the specification provides adequate testing and explanation to enable a skilled

artisan to make and use the invention commensurate in scope with the claimed invention of

methods for treating PTSD, disease states associated with increasing pH to improve fear

responses and CNS disorders characterized by a change in extracellular pH in the amygdala.

The specification sets forth for a skilled artisan a variety of *in vivo* testing, demonstrating

responses to fear conditioning effects (as representative of PTSD in humans) in mice, with

and without ASIC receptors. (*See, e.g.,* Specification p. 34, line 18 through p. 36, line 2).

Testing results and explanation of the materials and methods provide evaluation of fear

conditioning responses, which is defined in the specification as the "process of acquiring,

developing, educating, establishing, learning, or training responses in a patient having an

identifiable stimulus including, but not limited to, apprehension, dread or alarm."

(Specification p. 7, lines 1-3). The test results demonstrate that ASIC -/- mice have decreased reactions and response to fear conditioning, resulting in a decrease in behavior caused by fear. (Specification, FIGS. 8A-8D) (demonstrating ASIC -/- mice with decreased freezing responses compared to ASIC +/+ mice).

Such testing provides sufficient support for the enablement requirement that either: (1) drugs be given *in vivo*; (2) humans be tested; and/or (3) adequate animal models of PTSD and related stress and anxiety disorders be tested. Here, the testing set forth in the specification provides sufficient animal models for testing anxiety condition and diseases and disorders associated with changes in pH that are commensurate in scope with the claimed invention. Further, the Examiner has previously recognized and appreciated the *in vitro* testing where ASIC were indeed antagonized by the manipulation of surrounding pH, demonstrating the relationship between the ASIC receptor and disease states associated with increased pH and CNS disorders characterized by a change in extracellular pH in the amygdala. In light of such *in vivo* and *in vitro* testing provided in the specification and claims commensurate in scope with such testing, skilled artisans are able to make and use the claimed invention as required under 35 U.S.C. §112, first paragraph for the enablement requirement.

Secondly, the specification provides adequate testing and explanation to enable a skilled artisan to make and use the invention commensurate in scope with the claims directed to administering to patients in need of such treatment an ASIC antagonist. The specification is enabled for ASIC antagonists (agents capable of inhibiting the ASIC to improve fear

7

responses in patients) and not just the PcTx indicated as allowable by the Examiner. (12/4/08

Advisory Action). Although the Examiner's recognition that PcTx is one suitable ASIC

antagonist, the claimed invention is enabled for more than this agent alone.

The specification of the claimed invention discloses an entire family of ASIC cation

channels. (Specification p. 2, lines 4-20). The specification explains to skilled artisans that

ASIC are gated by reductions in pH and are related to amiloride-sensitive epithelial sodium

channels (ENaCs) and the degenerin/mec family of ion channels (DEGs). (Specification p.

12, lines 3-8). Additionally, distinct identifying characteristics of the ion channels are

provided to enable one of ordinary skill in the art to utilize any agent or composition capable

of antagonizing such ASIC. For example, the specification provides that subunits of the

DEG/ENaC protein family associate as homomultimers and heteromultimers to form voltage-

insensitive channels. (Specification p. 13, lines 10-28).

Moreover, the individual subunits share a common structure with two transmembrane

domains, intracellular carboxyl- and amino-termini, and a large, cysteine-rich extracellular

domain thought to serve as a receptor for extracellular stimuli. (Specification p. 13, lines 10-

28). Additionally, the specification provides for a specific antagonist of the DEG/ENaC

channels, the diuretic amiloride (FDA approved for treatment of hypertension, all

distinguishing chemical and compound characteristics known by one having ordinary skill in

the art), and the location and distribution of ASIC in regions of high synaptic plasticity such

as the lateral, basolateral and central nuclei of the amygdala that directly implicates the ASIC

receptors in the treatment of CNS disorders such as anxiety disorders. (Specification p. 13,

lines 13-16). The Examiner was also provided the disclosure of the Coryell *et al.* reference, disclosing additional ASIC antagonists including Psalmotoxin (PcTx) containing venom from the tarantula Psalmapoeus cambridget, containing a peptide antagonist of ASIC1a channels. (Coryell *et al.* p. 5). In addition, as the Examiner has recognized in indicating that the claims are enabled for the treatment of PTSD with PcTx, the precise antagonist is know and accessible by skilled artisans. For example, the sequencing of the peptide as determined by NMR spectroscopy is well known by those of ordinary skill in the art, making this yet another enabling example for the structure of additional ASIC antagonists to be made and used by such skilled artisans in order to practice the methods of the claimed invention. Accordingly, the specification sets forth various forms of antagonists that have been obtained and utilized by those of ordinary skill in the art for testing ASIC-related conditions and disorders that are commensurate in scope with the claimed invention.

Third, and finally, the specification provides adequate explanation to enable a skilled artisan to make and use the invention commensurate in scope with the claimed invention of administering, in a variety of administration routes, the ASIC antagonist. The Examiner's maintained enablement rejection according to the requirement that only direct administration to the brain of an ASIC antagonist is contrary to Patent Office practice. It is understood that skilled artisans are capable of providing a variety of delivery routes for medical treatments when a compound or drug of interest is identified. As the specification sets forth, beginning at p. 21, line 14 through p. 23, line 9, the various additions to the pharmaceutical compositions used in the claimed methods and the methods of delivery are well know to

9

skilled artisans. Therefore, it is unnecessary for the specification to contain precise details of

such modification of the claimed invention into every possible delivery method, including

oral, topical, sublingual, buccal, intranasal, rectal and intravenous. MPEP § 2164.01(c) (when

a statement of utility contains a connotation of how to use, and the art recognizes that

standard modes of administration (*i.e.* routes of administration), then 35 U.S.C. § 112 is

satisfied).

Accordingly, as the specification sets forth for skilled artisans the various conditions

requiring treatment under the claimed methods (including PTSD, disease states associated

with increased pH and CNS disorders characterized by a change in extracellular pH in the

amygdala), the ASIC antagonists and routes of administration for such methods, the

requirements of 35 U.S.C. § 112, first paragraph for enablement have been met by the

claimed invention.

B.   Claims 1-3, 24, 25, 30 and 31 have adequate written description under 35 U.S.C. § 112, first paragraph and are therefore patentable

1.   Legal Standard for Written Description

In rejecting claims under 35 U.S.C. §112, first paragraph, as lacking written

description, the Examiner must show that the subject matter of the claimed invention was not

described in such a way as to reasonably convey to one skilled in the relevant art that the

inventors, at the time the application was filed, had possession of the claimed invention.

Capon v. Eshhar, 418 F.3d 1349, 1357 (Fed. Cir. 2005). The requirement is necessary to

ensure that claimed inventions are adequately described in exchange for granting an applicant

10

the right to an issued patent. MPEP §2163. A specification can demonstrate possession of

the claimed invention, sufficient to meet the requirements under 35 U.S.C. §112, first

paragraph, in a variety of ways. *Lockwood v. American Airlines, Inc.,* 107 F.3d 1565, 1572

(Fed. Cir. 1997). (specification can use a variety of explanation, figures, diagrams, and

formulas for a written description).

Patent Office practice and case precedence requires that a fact-based inquiry be used

to determine whether the written description requirement is satisfied. *Enzo Biochem, Inc. v.*

Gen-Probe, Inc., 323 F.3d 956, 963 (Fed. Cir. 2002); MPEP §2163. Such an analysis varies

according to the type and nature of the claimed invention at issue. *Id.*

2. The Claims in Issues are Supported by a Written Description Satisfying 35 U.S.C. § 112, First Paragraph

The Examiner's rejection of claims 1-3, 24, 25, 30 and 31 under 35 U.S.C. § 112, first

paragraph, as containing subject matter which was not described in the specification in such a

way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time

the application was filed, had possession of the claimed invention is inconsistent with the

legal requirements for a written description. Similar to meeting the enablement requirement,

the specification (as applied to the written description requirement) also provides a showing

that, at the time of the application, the claimed invention was in possession of the various

uses for treatment with ASIC antagonists, the variety of ASIC antagonists that could be used

or determined by a skilled artisan to make and use, as well as the variety of routes of

11

administration for such treatments, all demonstrating the possession of the claimed invention at the time of filing the present invention.

The specification directed to the claimed inventions demonstrates that, at the time of filing the application, there was possession of the relationship between ASIC and various anxiety and fear response conditions (including PTSD, as recognized by the Examiner during prosecution of the appealed claims), testing demonstrating the treating of such conditions, the description of the ASIC and the various antagonists to be used with the ASIC for treatment as well as administration routes for such treatment. These teachings in the specification meet the requirements under 35 U.S.C. § 112, first paragraph, as applied to the written description requirement.

The Examiner acknowledged that the specification discloses experiments confirming the role of ASIC1 receptors in fear conditioning and short-term memory. Additionally, the Examiner recognized the relationship between ASIC and the need for treatment relating to PTSD (an anxiety-related condition) and disease states associated with increased pH and CNS disorders characterized by a change in extracellular pH in the amygdala, which also relate to the need for improved fear responses. In all appealed claims, the causation of improved fear responses is a necessary element of treating PTSD, as identified as a supported element by the Examiner. The effect of ASIC receptors on fear responses, as set forth in the above-described fear conditioning examples, has been acknowledged by the Examiner as being a condition involving the ASIC receptor.

Additionally, the claimed invention highlights the necessity for improved fear responses in order to treat PTSD. Such fear responses were tested according to the claimed invention *in vivo* in mice and therefore obviates the Examiner's concern that skilled artisans might not "envision the detailed methods needed to treat an anxiety disorder by administering an ASIC antagonist." (8/20/08 Final Rejection Office Action, p. 8). However, the specification shows animal model testing with positive improvements sufficient for predicting related success in humans, according to approaches set forth in the originally-filed specification. (Specification p. 26, lines 9-31). There, the specification describes the methods incorporated by reference into the specification under the "Materials and Methods" portion that predict experimental success in humans based on animal models, such as the ASIC knockout mice generated by homologous recombination in embryonic stem cells. (Specification p. 26, lines 9-12).

Finally, the specification rebuts the Examiner's statement that an insufficient number of compounds inhibiting the ASIC1a are found in the methods. (12/4/08 Advisory Action). As discussed with regard to the enablement requirement, the specification provides sufficient testing and explanation related to the claimed invention, specifically the treatment methods using an ASIC antagonist. The specification is enabled for all ASIC antagonists, in addition to the precise antagonists identified in the specification. The claimed invention has sufficient written description for more than the PcTx ASIC antagonist alone.

The specification discloses an entire family of ASIC cation channels gated by reductions in pH and are related to amiloride-sensitive epithelial sodium channels (ENaCs)

and the degenerin/mec family of ion channels (DEGs). (Specification p. 2, lines 4-20). Distinct characteristics of the ASIC are also provided in the description for further explanation of the agents or compositions necessary to antagonize such channels, including: subunits of the DEG/ENaC protein family associating as homomultimers and heteromultimers to form voltage-insensitive channels (Specification p. 13, lines 10-28); individual subunits with two transmembrane domains, intracellular carboxyl- and amino-termini, and a large, cysteine-rich extracellular domain thought to serve as a receptor for extracellular stimuli (Specification p. 13, lines 10-28); and an examples of specific antagonists of the DEG/ENaC channels, including the diuretic amiloride (Specification p. 13, lines 13-16) and PcTx (Specification p. 14, lines 6-10).

Accordingly, the specification, as originally-filed at the time of the application, sets forth various forms of antagonists that have been obtained and utilized by those of ordinary skill in the art, for testing ASIC-related conditions and disorders that are commensurate in scope with the claimed invention. Therefore, claims 1-3, 24, 25, 30 and 31 are supported by a specification that demonstrates possession of the claimed invention, sufficient to meet the requirements under 35 U.S.C. §112, first paragraph, for written description.

IX.   CONCLUSION

For the above-stated reasons, it is submitted that the claims are in a condition for allowability. The decision of the Examiner, therefore, should be reversed and the case allowed.

Please charge Deposit Account No. 26-0084 the amount of $540.00 for the large

entity fee for the Appeal Brief. No other fees or extensions of time are believed to be due in

connection with this brief; however, consider this a request for any extension inadvertently

omitted, and charge any additional fees to Deposit Account No. 26-0084.

Respectfully submitted,

JILL N. LINK, Reg. No. 62,123
McKEE, VOORHEES & SEASE
801 Grand Avenue, Suite 3200
Des Moines, Iowa 50309-2721
Phone No: (515) 288-3667
Fax No: (515) 288-1338
Attorneys of Record
CUSTOMER NO. 22885

X. APPENDIX - CLAIMS

1.   A method of treatment for post-traumatic stress disorder comprising:

administering to a patient in need thereof a therapeutically effective amount of an acid

sensing ion channel (ASIC) antagonist and a pharmaceutically acceptable carrier,

wherein said antagonist causes improved fear responses.

2.   The method of claim 1 wherein the ASIC antagonist is an acid-sensing ion channel 1

(ASIC1) receptor antagonist.

3.   The method according to claim 1 wherein said acid sensing ion channel (ASIC)

antagonist and said pharmaceutically acceptable carrier is administered by a route selected

from the group consisting of: orally, topically, sublingually, buccally, intranasally, rectally

and intravenously.

24.   A method of treating a disease state associated with increased pH which comprises:

administering to a patient a therapeutically effective amount of an acid sensing ion channel

(ASIC) antagonist capable of improving fear responses and a pharmaceutically

acceptable carrier.

25.   The method of claim 24 wherein the disease state is the anxiety condition

of post-traumatic stress disorder.

30.   A method of treating a CNS disorder characterized by a change in extracellular pH in

the amygdala comprising:

inhibiting the acid-sensing ion channel 1 (ASIC1) in order to improve fear responses in a

        patient in need of such treatment.

31.   The method of claim 30 wherein said CNS disorder is post-traumatic stress disorder.

X.   EVIDENCE APPENDIX

Only evidence of record has been relied upon in this appeal.

<u>Exhibit A</u>: Coryell *et al.* (2007), Targeting ASIC1a Reduces Innate Fear and Alters Neuronal Activity in the Fear Circuit, *Biol. Psychiatry.*, 62(10):1140-8.

The reference was entered by the Examiner pursuant to Applicants' Information Disclosure Statement submitted November 4, 2008 and considered by the Examiner on December 1, 2008.

XI. RELATED PROCEEDING APPENDIX

None.

Targeting ASIC1a Reduces Innate Fear and Alters Neuronal Activity in the Fear Circuit

Matthew W. Coryell, Adam E. Ziemann, Patricia J. Westmoreland, Jill M. Haenfler, Zlatan Kurjakovic, Xiang-ming Zha, Margaret Price, Mikael K. Schnizler, and John A. Wemmie

Background: The molecular mechanisms underlying innate fear are poorly understood. Previous studies indicated that the acid sensing ion channel ASIC1a influences fear behavior in conditioning paradigms. However, these differences may have resulted from an ASIC1a effect on learning, memory, or the expression of fear.

Methods: To test the hypothesis that ASIC1a influences the expression of fear or anxiety independent of classical conditioning, we examined the effects of disrupting the mouse *ASIC1a* gene on unconditioned fear in the open field test, unconditioned acoustic startle, and fear evoked by the predator odor trimethylthiazoline (TMT). In addition, we tested the effects of acutely inhibiting ASIC1a with PcTx, an ASIC1a antagonist in tarantula venom. Our immunohistochemistry suggested ASIC1a is expressed in the bed nucleus of the stria terminalis, medial amygdala, and periaqueductal gray, which are thought to play important roles in the generation and expression of innate fear. Therefore, we also tested whether ASIC1a disruption altered c-fos expression in these structures following TMT exposure.

Results: We found that the loss of ASIC1a reduced fear in the open field test, reduced acoustic startle, and inhibited the fear response to TMT. Similarly, intracerebroventricular administration of PcTx reduced TMT-evoked freezing in ASIC1a$^{+/+}$ mice but not ASIC1a$^{-/-}$ mice. In addition, loss of ASIC1a altered TMT-evoked c-fos expression in the medial amydala and dorsal periaqueductal gray.

Conclusions: These findings suggest that ASIC1a modulates activity in the circuits underlying innate fear. Furthermore, the data indicate that targeting the ASIC1a gene or acutely inhibiting ASIC1a suppresses fear and anxiety independent of conditioning.

While some fears are learned through association, humans and animals may also be biologically or innately predisposed to fear some stimuli (1–4). This predisposition may contribute to neuroticism and the development of anxiety disorders (3,4). Thus, understanding the mechanisms of innate fear in animal models may provide valuable insight into the pathogenesis of anxiety disorders.

Innate (or unconditioned) fear is poorly understood, however some required brain regions have been identified in rodents. Inactivating the bed nucleus of the stria terminalis (BNST) (5) or medial amygdala (6) attenuated predator odor-evoked fear, and other forms of unconditioned fear (5,7). Thus, it has been suggested that unconditioned fear may rely on brain circuits different from those required for fear conditioning such as the lateral, basolateral, and central amygdala (8,9). However, evidence also suggests overlap between conditioned and unconditioned fear circuits. For example, inactivating the bed nucleus of the stria terminalis (10) and medial amygdala (11) also attenuated context fear conditioning. Furthermore, inactivating the basolateral amygdala (BLA) inhibited unconditioned fear (6,12) in addition to suppressing the acquisition, retention, and expression of fear conditioning (for review [13]). Beyond the anatomic basis for unconditioned fear, relatively little is known about the molecular mechanisms underlying innate fear.

A molecule that might contribute to innate fear is the acid sensing ion channel ASIC1a. ASIC1a is a recently discovered member of the degenerin/epithelial Na$^+$ channel family that is activated by extracellular acidosis and is expressed in the amygdala complex and elsewhere in the brain (14–17). ASIC1a is required for acid-evoked currents in central neurons, and is located at excitatory synapses (14), where it modulates synaptic Ca^{2+} signaling (18,19) and synaptic plasticity (14, 20–22). ASIC1a enables dendritic spines to respond to acid pH (18), suggesting synaptically released protons might activate it (23). ASIC1a can be modulated by N-methyl-D-aspartate (NMDA)-receptor activation (19), and the loss of ASIC1a disrupted NMDA-receptor dependent long-term potentiation and temporal summation of excitatory postsynaptic potentials (14). Consistent with a role in fear learning and memory, deleting ASIC1a reduced fear in conditioning paradigms (17), while overexpressing ASIC1a increased context fear conditioning (24). However, it is not clear to what degree the ASIC1a-dependent effects were due to differences in fear acquisition, fear retention, or fear expression.

To test the effects of ASIC1a on the expression of fear and anxiety, we examined the impact of disrupting the *ASIC1a* gene or acutely blocking ASIC1a on mouse models of unconditioned fear, including fear of open spaces, unconditioned acoustic startle, and fear evoked by the predator odor trimethylthiazoline (TMT) (7,8,25–28). To explore whether differences were due to sensory or motor dysfunction, we also tested the effects of ASIC1a on hearing, footshock evoked startle, and olfactory dependent behaviors. Finally, we examined ASIC1a distribution in forebrain and midbrain structures required for TMT-evoked fear, and tested whether loss of ASIC1a altered c-fos induction in these structures.

From the Neuroscience Program (MWC, JAW), Departments of Psychiatry (MWC, JMH, ZK, JAW), Internal Medicine (PJW, XZ, MP, MKS), and Molecular Physiology and Biophysics (AEZ), Roy J. and Lucille A. Carver College of Medicine, University of Iowa; Department of Veterans Affairs Medical Center (MWC, JMH, JAW), Iowa City, Iowa.

Address reprint requests to John Wemmie, M.D., Ph.D., Department of Psychiatry, University of Iowa College of Medicine, 500 Eckstein Medical Research Building, Iowa City, IA 52242; E-mail: john-wemmie@uiowa.edu.

Received March 14, 2007; revised April 27, 2007; accepted May 10, 2007.

EXHIBIT

tabbies

_____A_____

quantified by an investigator blinded to genotype using Image J software (National Institutes of Health, Bethesda, Maryland) as described (32). The anatomical boundaries for the structures tested were defined based on the Paxinos atlas (33). For amygdala nuclei (central, lateral [LA], and medial) sections were selected from between 1.7 and 1.6 mm caudal to bregma. For the BNST and piriform cortex, sections were collected from between .14 and .28 mm rostral to bregma. For periaqueductal gray (PAG) analysis, sections were collected from between 4.48 and 4.60 mm caudal to the bregma.

Results

Loss of ASIC1a Reduces Fear-Behavior in the Open Field

To test whether ASIC1a disruption affects innate fear, we assessed fear in the open field by measuring infrared beam breaks. Naive mice fear open spaces (27), likely because of the associated risk of predation. Thus, in this test mice usually avoid the center, preferring to move around the edges. Consistent with a fear of open spaces, we found that both wild-type and ASIC1a-null mice avoided the center. However, ASIC1a-null mice had significantly more center beam breaks than wild-type controls (Figure 1A). Total beam breaks were similar between the two genotypes (Figure 1B), suggesting that loss of ASIC1a does not affect motor activity overall. These data suggest that the loss of ASIC1a reduces fear of open spaces.

ASIC1a Disruption Reduces Unconditioned Acoustic Startle, but Does Not Affect Hearing

The startle response to sudden noise is thought to reflect emotional arousal and is increased in patients with anxiety disorders (34–36). Particularly in mice, baseline startle may be a valuable measure of anxiety (25). Therefore, we hypothesized that if ASIC1a contributes to innate fear, then disrupting ASIC1a may attenuate unconditioned acoustic startle. To test this hypothesis, we used an approach similar to that described by others (25); we placed naive wild-type and ASIC1a-null mice into an acoustic startle chamber and assessed the average maximal startle amplitude evoked by 10 presentations of white noise (115 dB). Consistent with reduced innate fear, the ASIC1a$^{-/-}$ mice startled significantly less than ASIC1a$^{+/+}$ mice (Figure 1C, 1D). In addition, the differences between the two groups did not vary significantly by trial number (Figure 1C), suggesting the effect of ASIC1a on acoustic startle was probably not due to altered sensitization or habituation.

To test whether the blunted acoustic startle in the ASIC1a$^{-/-}$ mice might be caused by hearing loss, we also assessed EABR. We found no differences in the EABR attenuation threshold between ASIC1a$^{+/+}$ and ASIC1a$^{-/-}$ mice (Figure 1E), suggesting hearing is normal in the ASIC1a-null mice. We also examined whether ASIC1a disruption might impair motor reflexes. We assessed startle responses to a footshock (.7 mA), which is well above the threshold of detection (17,24). Because footshock activates peripheral sensory and nociceptive fibers, spinal reflexes are likely to play a large role in this behavior (37). We found ASIC1a$^{+/+}$ and ASIC1a$^{-/-}$ mice produced a similar response to footshock (ASIC1a$^{+/+}$ amplitude = 1774.5 ± 247. (SEM); ASIC1a$^{-/-}$ amplitude = 1645.8 ± 201.7 (SEM); n = 5 per group; df (8), t = .37, p = .74). This result is consistent with previous indications that sensory responses and motor function are grossly intact in the ASIC1a$^{-/-}$ mice (14,17,38,39).
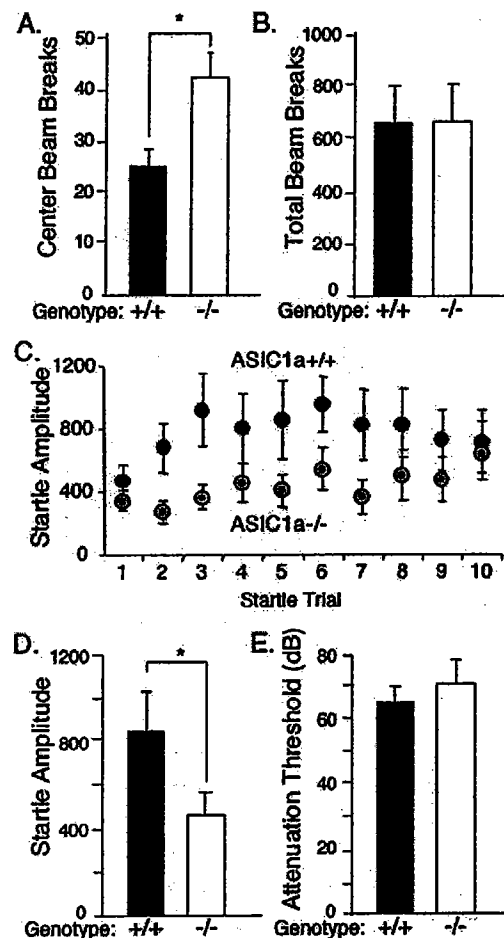

Figure 1. ASIC1a disruption reduces center-avoidance in the open field and reduces acoustic startle. (A) Center avoidance was measured by center beam breaks. ASIC1a$^{-/-}$ mice had significantly more center beam breaks than ASIC1a$^{+/+}$ mice ($p < .004$; +/+, n = 13; –/–, n = 16). (B) Total activity in the open field was similar between genotypes (df (30), t = .04, $p > .96$; +/+, n = 9; –/–, n = 12). (C) Unconditioned acoustic startle amplitude separated by trial number. There was no significant genotype × trial interaction (+/+, n = 9; –/–, n = 12), (df (1,9), F = 1.43, p = .18) suggesting the differences between the genotypes did not vary significantly between trials. (D) Amplitude of acoustic startle responses pooled from 10 trials was reduced in the ASIC1a$^{-/-}$ mice relative to ASIC1a$^{+/+}$ controls (df(19), t = 2.00, p = .029). (E) Attenuation threshold of the evoked auditory brainstem response did not differ between ASIC1a$^{+/+}$ and ASIC1a$^{-/-}$ mice (df(11), t = –1.38, p = .20; +/+, n = 6; –/–, n = 7).

Loss of ASIC1a Disrupts Unconditioned Responses to the Predator Odor TMT

As another model of innate fear we assessed TMT-evoked fear behaviors. TMT is a sulfur-containing compound isolated from the anal gland of foxes (40) and previously shown by others to evoke BNST-dependent unconditioned freezing in rodents (5,41–43). Consistent with those studies, TMT evoked significant freezing in ASIC1a$^{+/+}$ mice. In contrast, ASIC1a$^{-/-}$ mice froze significantly less in response to TMT (Figure 2A, 2B) and see Supplement 1 online). Consistent with the previous suggestion that female C57Bl6 mice may have greater predator odor-evoked fear (44), wild-type females froze more than their male counterparts (Figure 2C). However, the loss of ASIC1a reduced freezing in both genders. In addition to their reduced freezing response, the ASIC1a$^{-/-}$ mice avoided contact with the TMT less than wild-type

Acute Inhibition of ASIC1a Decreases TMT-Evoked Freezing

Our data indicate that genetically disrupting ASIC1a reduces unconditioned fear. This might be due to a requirement for ASIC1a during fear behavior or a result of abnormal development. To distinguish between these possibilities we tested whether acute inhibition of ASIC1a could reduce fear behavior using PcTx venom from the tarantula *Psalmapoeus cambridgei*. Previous studies indicated that PcTx venom contains a peptide antagonist of ASIC1a channels (46) and that ICV infusion of PcTx reduces acid-associated toxicity during stroke (16). Consistent with previous studies (16,47), in cultured cortical neurons from ASIC1a$^{+/+}$ mice, PcTx (1.8 ng/μL) blocked 64% ($\pm$ 6% SEM, $n = 3$) of the average peak pH 6-evoked current (Figure 4A). Because PcTx only blocks ASIC1a homomultimeric channels, the remaining current is likely to be due to ASIC1a-containing heteromultimers with ASIC2, as suggested previously (47). We hypothesized that a similar dose of PcTx venom (5 μl, 9 ng/mL) in ACSF would reduce unconditioned fear of the predator odor TMT. ICV administration of PcTx venom reduced TMT-evoked freezing in wild-type mice, but did not affect TMT-evoked freezing in ASIC1a-null mice (Figure 4B). The ASIC1a-null mice provided an important control for nonspecific effects of the venom. These results suggest that acutely inhibiting ASIC1a reduces unconditioned fear. In addition, these data rule out a

developmental requirement for ASIC1a in innate fear, and suggest that innate fear is closely tied to ASIC1a function.

ASIC1a is Abundant in the BNST, Amygdala, and Periaqueductal Gray

Earlier studies suggested that ASIC1a is enriched in the amygdala (17), including the medial nucleus which is required for predator odor evoked fear (26). However, it is not known whether ASIC1a is also expressed in the BNST or PAG which play critical roles in the expression of innate fear, freezing, and other defensive behaviors (7,48–54). Therefore, to assess ASIC1a distribution in these key structures, we used immunohistochemistry to test ASIC1a expression in the forebrain and midbrain. ASIC1a$^{-/-}$ mice provided a valuable control for ASIC1a-specific immunostaining. Relative to other structures, we found ASIC1a immunostaining was strikingly abundant in the BNST, BLA, and PAG of ASIC1a$^{+/+}$ mice (Figure 5A, 5B, 5C). Consistent with our previous results (17), ASIC1a was also present in the lateral, medial, and central amygdala nuclei as well as the cingulate cortex, habenula, and lateral hypothalamus (Figure 5A). These data suggest ASIC1a may contribute to innate fear by influencing neuronal activity in fear circuit structures.

Loss of ASIC1a Alters TMT Induced c-fos Expression in the Medial Amygdala and Dorsal Periaqueductal Gray

To assess whether the differences in fear behavior were associated with changes in fear circuit activity, we assessed expression of the immediate early gene c-fos as a marker of neuron activation in key fear circuit structures following TMT exposure. We hypothesized that ASIC1a disruption would alter c-fos expression in the medial amygdala, BNST and PAG; regions where ASIC1a is abundant and that are critical for TMT-evoked fear (5,6,50,55–57). We also assessed c-fos expression in the LA and BLA because ASIC1a is abundant in these structures (17). However, the importance of the LA and BLA in TMT-evoked fear is less certain (6,8,43). Following TMT exposure, we detected ASIC1a-dependent differences in both the medial amygdala and dorsal PAG (Figure 6). In the medial amygdala, the loss of ASIC1a attenuated c-fos induction (Figure 6A, 6B, 6E). Interestingly, in the dorsal PAG (dPAG), TMT produced differential effects in ASIC1a$^{+/+}$ mice compared to ASIC1a$^{-/-}$ mice. In ASIC1a$^{+/+}$ mice, TMT reduced c-fos expression in the dPAG; whereas in the ASIC1a$^{-/-}$ mice, TMT marginally increased c-fos expression (Figure 6E). Because of the importance of the medial amygdala and PAG in freezing and defense behaviors, these differences are likely to contribute to the reduced fear behaviors in the ASIC1a$^{-/-}$ mice.

In contrast, we found no significant ASIC1a-dependent differences in the BNST, ventral PAG, BLA or LA following TMT exposure (Figure 6F). This could mean that ASIC1a in these regions does not contribute to TMT-evoked fear. It is also possible that c-fos expression is not sensitive to ASIC1a-dependent effects in these regions.

Discussion

Relatively little is known about the molecular underpinnings of innate fear (7,8), however, our results suggest that ASIC1a plays a critical role. In naive mice, loss of the *ASIC1a* gene or acute ASIC1a inhibition reduced fear-related behaviors evoked by multiple stimuli and measured by multiple behavioral outputs, including avoidance, startle, and freezing. Together these data suggest that ASIC1a plays a central role in innate fear, and that
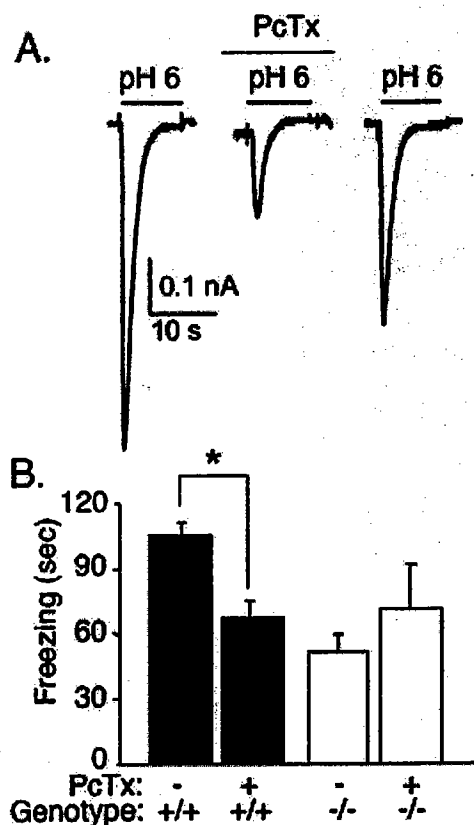

Figure 4. Inhibiting ASIC1a with PcTx reduces the freezing reponse to TMT. **(A)** Consistent with previous studies (16, 47), PcTx (9 ng/μL) inhibited acid-evoked currents in cultured cortical neurons. Elapsed time between traces was 110 and 160 sec respectively. **(B)** ICV infusion of PcTx significantly reduced TMT-evoked freezing during a 5 min trial in ASIC1a$^{+/+}$ mice ($df = 26.9, t = -3.31, *p = .0027; n = 12$) but not in ASIC1a$^{-/-}$ mice ($df = 26.9, t = 1.29, p > .20; n = 7$). ICV, intracerebroventricular; TMT, trimethylthiazoline.

A. ASIC1a$^{+/+}$ (+TMT)
B. ASIC1a$^{-/-}$ (+TMT)

medial amygdala

C. ASIC1a$^{+/+}$ (+TMT)
D. ASIC1a$^{-/-}$ (+TMT)

dorsal PAG

E. c-fos positive nuclei/mm^2

TMT: - - + + - - + +
Genotype: +/+ -/- +/+ -/- +/+ -/- +/+ -/-
medial amygdala / dorsal PAG

F. c-fos positive nuclei/mm^2

TMT: + + + + + + + +
Genotype: +/+ -/- +/+ -/- +/+ -/- +/+ -/-
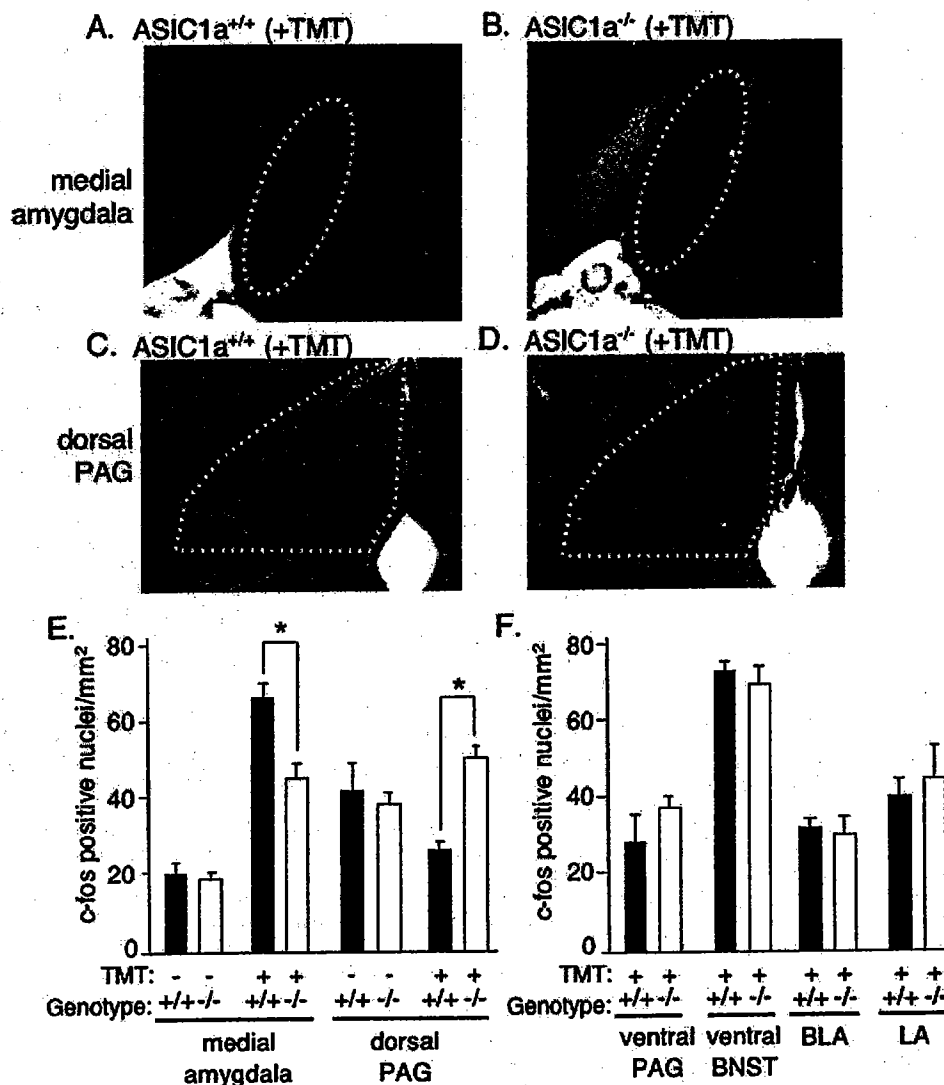ventral PAG / ventral BNST / BLA / LA

Figure 6. Loss of ASIC1a alters TMT induction of c-fos in the medial amygdala and dPAG. (A, B) Examples of c-fos expression in the medial amygdala following TMT exposure from ASIC1a$^{+/+}$ and ASIC1a$^{-/-}$ mice. (C, D) Examples of c-fos expression in the dPAG following TMT exposure. (E) Quantification revealed a significant increase in c-fos expression in medial amygdala following TMT exposure (df (1, 17.5), $F = 128.85, p < .0001$) as well as a significant genotype × TMT interaction (df (1, 17.5), $F = 8.82, p = .0084$; No odor (ASIC1a$^{+/+}$, $n = 5$; ASIC1a$^{-/-}$, $n = 5$); TMT (ASIC1a$^{+/+}$, $n = 10$; ASIC1a$^{-/-}$, $n = 9$)). In the dPAG, TMT decreased c-fos in the dPAG of ASIC1a$^{+/+}$ mice ($df = 15, t = -2.91, p = .011$) and increased c-fos expression in the dPAG of ASIC1a$^{-/-}$ mice, although the p value was marginal ($df = 19, t = -1.99, p = .06$). Consequently, following TMT exposure c-fos expression in the dPAG was significantly greater in ASIC1a$^{-/-}$ mice relative to ASIC1a$^{+/+}$ mice ($df = 28, t = -6.40, p < .0001$), resulting in a significant genotype × TMT interaction (df (1, 34), $F = 11.13, p = .002$; No odor (ASIC1a$^{+/+}$, $n = 4$; ASIC1a$^{-/-}$, $n = 4$); TMT (ASIC1a$^{+/+}$, $n = 13$; ASIC1a$^{-/-}$, $n = 17$). (F) In the BNST, vPAG, BLA, and LA there were no significant differences between genotypes in c-fos expression following TMT exposure (BNST: $df = 6.66, t = .68, p > .51$; ASIC1a$^{+/+}$, $n = 6$; ASIC1a$^{-/-}$, $n = 6$) (vPAG: $df = 7, t = -1.2, p = .24$; ASIC1a$^{+/+}$, $n = 4$; ASIC1a$^{-/-}$, $n = 5$) (BLA: $df = 10, t = .22, p > .83$; $n = 6$ per group) (LA: $df = 10, t = -.59, p > .56$; $n = 6$ per group). BLA, basolateral amygdala; BNST, bed nucleus of the stria terminalis; dPAG, dorsal periaqueductal gray; LA, lateral; TMT, trimethylthiazoline; vPAG, ventral preiaqueductal gray.

tures. Second, the neural circuitry of the medial amygdala and PAG are complex (50,53); making it difficult to know whether the ASIC1a-dependent differences occur in excitatory or inhibitory pathways.

Others have previously examined c-fos induction by TMT (55,61) and cat odor (62). In general, those studies found c-fos was increased at multiple locations in the fear circuit; although some differences between TMT and cat odor have been pointed out (26,28) which could reflect differences between specific odors, odor concentration, or other testing conditions. Our data are consistent with the suggestion that predator odors increase c-fos in the fear circuit; particularly in the ventral BNST and the medial amygdala (55,62). Interestingly, cat odor was previously suggested to increase c-fos in the dPAG (62), while in our studies TMT suppressed c-fos in the dPAG of wild type mice. This difference could be due to the testing conditions. In our studies the mice were unable to escape TMT and exhibited robust freezing, while in the previous study rats were given a hide box to escape the cat odor (62). These observations are consistent with the previously observed role of the dPAG in escape (53), and with others' data suggesting an inverse correlation between freezing and c-fos expression in the dPAG (63). Thus, the altered

activities in the medial amygdala and dPAG observed here are likely to contribute to the reduced fear and reduced freezing in the ASIC1a$^{-/-}$ mice.

Based on these studies, one might expect ASIC1a to affect anxiety-related behavior in the elevated plus maze (EPM). Curiously, open arm crossings and risk assessing behaviors in a 1-trial EPM paradigm were previously shown to be unaffected by ASIC1a-disruption (17). Because EPM can be sensitive to a number of factors including handling, lighting, and genetic background (64), it is possible that small ASIC1a-dependent effects on EPM may have been missed (17). Alternatively, ASIC1a may not contribute to this behavior. Others have noted that 1-trial EPM is not universally sensitive to manipulations that affect fear, including basolateral amygdala lesions (65), and altered neuropeptide signaling (64).

Here we found that the loss of ASIC1a reduces fear behaviors thought to occur independent of associative learning. Hence, our results suggest that ASIC1a modulates fear circuit activity independent of its role in synaptic plasticity (14). Perhaps the previously observed effects of ASIC1a on conditioned fear tasks (17,24) are due to differences in fear expression rather than fear conditioning. It is interesting to note that AMPA receptor block-

31. Dominguez-Salazar E, Bateman HL, Rissman EF (2004): Background matters: The effects of estrogen receptor alpha gene disruption on male sexual behavior are modified by background strain. *Horm Behav* 46: 482–490.

32. Grippo AJ, Na ES, Johnson RF, Beltz TG, Johnson AK (2004): Sucrose ingestion elicits reduced Fos expression in the nucleus accumbens of anhedonic rats. *Brain Res* 1019:259–264.

33. Paxinos G, Franklin KBJ (2001): The Mouse Brain in Steriotaxic Coordinates, 2nd ed. San Diego: Academic Press.

34. Butler RW, Braff DL, Rausch JL, Jenkins MA, Sprock J, Geyer MA (1990): Physiological evidence of exaggerated startle response in a subgroup of Vietnam veterans with combat-related PTSD. *Am J Psychiatry* 147:1308–1312.

35. Grillon C, Merikangas KR, Dierker L, Snidman N, Arriaga RI, Kagan J, et al. (1999): Startle potentiation by threat of aversive stimuli and darkness in adolescents: a multi-site study. *Int J Psychophysiol* 32:63–73.

36. Grillon C, Baas J (2003): A review of the modulation of the startle reflex by affective states and its application in psychiatry. *Clin Neurophysiol* 114:1557–1579.

37. Le Bars D, Gozariu M, Cadden SW (2001): Animal models of nociception. *Pharmacol Rev* 53:597–652.

38. Sluka KA, Price MP, Wemmie JA, Welsh MJ (2003): ASIC3, but not ASIC1, channels are involved in the development of chronic muscle pain, in Proceedings of the 10th World Congress on Pain, Progress in Pain Research and Management, O. Dostrovsky et al., Editors. IASP Press: Seattle. p. 71–79.

39. Page AJ, Brierley SM, Martin CM, Martinez-Salgado C, Wemmie JA, Brennan TJ, et al. (2004): The ion channel ASIC1 contributes to visceral but not cutaneous mechanoreceptor function. *Gastroenterology* 127:1739–1747.

40. Vernet-Maury E (1980): *Trimethyl-thiazoline in Fox Feces: A Natural Alarming Substance for the Rat.* In: van der Starre H, ed. Olfaction and Taste, Volume 7. Washington, DC: IRL Press.

41. Hotsenpiller G, Williams JL (1997): A synthetic predator odor (TMT) enhances conditioned analgesia and fear when paired with a benzodiazepine receptor inverse agonist (FG-7142). *Psychobiology* 25:83–88.

42. Wallace KJ, Rosen JB (2000): Predator odor as an unconditioned fear stimulus in rats: Elicitation of freezing by trimethylthiazoline, a component of fox feces. *Behav Neurosci* 114: 912–922.

43. Wallace KJ, Rosen JB (2001): Neurotoxic lesions of the lateral nucleus of the amygdala decrease conditioned fear but not unconditioned fear of a predator odor. Comparison with electrolytic lesions. *J Neurosci* 21: 3619–2627.

44. Adamec R, Head D, Blundell J, Burton P, Berton O (2006): Lasting anxiogenic effects of feline predator stress in mice: Sex differences in vulnerability to stress and predicting severity of anxiogenic response from the stress experience. *Physiol Behav* 88:12–29.

45. Yokosuka M, Matsuoka M, Ohtani-Kaneko R, Iigo M, Hara M, Hirata K, et al. (1999): Female-soiled bedding induced fos immunoreactivity in the ventral part of the premammillary nucleus (PMv) of the male mouse. *Physiol Behav* 68:257–261.

46. Escoubas P, De Weille JR, Lecoq A, Diochot S, Waldmann R, Champigny G, et al. (2000): Isolation of a tarantula toxin specific for a class of proton-gated Na+ channels. *J Biol Chem* 275:25116–25121.

47. Baron A, Waldmann R, Lazdunski M (2002): ASIC-like, proton-activated currents in rat hippocampal neurons. *J Physiol* 539:485–494.

48. Lee Y, Davis M (1997): Role of the hippocampus, the bed nucleus of the stria terminalis, and the amygdala in the excitatory effect of corticotropin-releasing hormone on the acoustic startle reflex. *J Neurosci* 17:6434–6446.

49. Fendt M, Siegl S, Steiniger-Brach B (2005): Noradrenaline transmission within the ventral bed nucleus of the stria terminalis is critical for fear behavior induced by trimethylthiazoline, a component of fox odor. *J Neurosci* 25:5998–6004.

50. Vianna DM, Brandao ML (2003): Anatomical connections of the periaqueductal gray: Specific neural substrates for different kinds of fear. *Braz J Med Biol Res* 36:557–566.

51. Vianna DM, Carrive P (2005): Changes in cutaneous and body temperature during and after conditioned fear to context in the rat. *Eur J Neurosci* 21:2505–2512.

52. Leman S, Dielenberg RA, Carrive P (2003): Effect of dorsal periaqueductal gray lesion on cardiovascular and behavioural responses to contextual conditioned fear in rats. *Behav Brain Res* 143:169–176.

53. Bandler R, Shipley MT (1994): Columnar organization in the midbrain periaqueductal gray: Modules for emotional expression? *Trends Neurosci* 17:379–389.

54. LeDoux JE (2000): Emotion Circuits in the Brain. *Annu Rev Neurosci* 23: 155–184.

55. Day HE, Masini CV, Campeau S (2004): The pattern of brain c-fos mRNA induced by a component of fox odor, 2,5-dihydro-2,4,5-trimethylthiazoline (TMT), in rats, suggests both systemic and processive stress characteristics. *Brain Res* 1025:139–151.

56. Farook JM, Wang Q, Moochhala SM, Zhu ZY, Lee L, Wong PT (2004): Distinct regions of periaqueductal gray (PAG) are involved in freezing behavior in hooded PVG rats on the cat-freezing test apparatus. *Neurosci Lett* 354:139–142.

57. Graeff FG, Silveira MC, Nogueira RL, Audi EA, Oliveira RM (1993): Role of the amygdala and periaqueductal gray in anxiety and panic. *Behav Brain Res* 58:123–131.

58. Bianchi L, Driscoll M (2002): Protons at the gate: DEG/ENaC ion channels help us feel and remember. *Neuron* 34:337–340.

59. Dielenberg RA, McGregor IS (2001): Defensive behavior in rats towards predatory odors: A review. *Neurosci Biobehav Rev* 25:597–609.

60. Rosen JB, Fanselow MS, Young SL, Sitcoske M, Maren S (1998): Immediate-early gene expression in the amygdala following footshock stress and contextual fear conditioning. *Brain Res* 796:132–142.

61. Hebb AL, Zacharko RM, Dominguez H, Trudel F, Laforest S, Drolet G (2002): Odor-induced variation in anxiety-like behavior in mice is associated with discrete and differential effects on mesocorticolimbic cholecystokinin mRNA expression. *Neuropsychopharmacology* 27:744–755.

62. Dielenberg RA, Hunt GE, McGregor IS (2001): "When a rat smells a cat": the distribution of Fos immunoreactivity in rat brain following exposure to a predatory odor. *Neuroscience* 104:1085–1097.

63. Lamprea MR, Cardenas FP, Vianna DM, Castilho VM, Cruz-Morales SE, Brandao ML (2002): The distribution of fos immunoreactivity in rat brain following freezing and escape responses elicited by electrical stimulation of the inferior colliculus. *Brain Res* 950:186–194.

64. Carobrez AP, Bertoglio LJ (2005): Ethological and temporal analyses of anxiety-like behavior: the elevated plus-maze model 20 years on. *Neurosci Biobehav Rev* 29:1193–1205.

65. Treit D, Menard J (1997): Dissociations among the anxiolytic effects of septal, hippocampal, and amygdaloid lesions. *Behav Neurosci* 111:653–658.

66. Blanchard DC, Blanchard RJ, Carobrez Ade P, Veniegas R, Rodgers RJ, Shepherd JK (1992): MK-801 produces a reduction in anxiety-related antipredator defensiveness in male and female rats and a gender-dependent increase in locomotor behavior. *Psychopharmacology (Berl)* 108:352–362.

67. Venton BJ, Robinson TE, Kennedy RT (2006): Transient changes in nucleus accumbens amino acid concentrations correlate with individual responsivity to the predator fox odor 2,5-dihydro-2,4,5-trimethylthiazoline. *J Neurochem* 96:236–246.

68. Smoller JW, Acierno JS Jr, Rosenbaum JF, Biederman J, Pollack MH, Meminger S, et al. (2001): Targeted genome screen of panic disorder and anxiety disorder proneness using homology to murine QTL regions. *Am J Med Genet* 105:195–206.